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A D DIMER BIOSENSOR TO MONITOR STROKE PATIENTS DURING THROMBOLYTIC THERAPY

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Introduction

We have developed a D dimer biosensor which can be use to monitor stroke patients during thrombolytic therapy. In the United States, thromboembolic strokes account for an estimated 300,000-400,000 of the 550,000 reported new cases of stroke each year (1). These strokes can be treated using thrombolytic therapy.

Thrombolytic therapy is a minimally invasive procedure that involves using a steerable microguidewire to thread a microcatheter to the site of the occlusion. Pharmacological agents are then directly injected into the occlusion to lyse the clot. The thrombus then dissolves into fragments. A particular diagnostic fragment is called the D dimer fragment which has antigenic properties. Thrombolytic therapy is highly effective; however, optimal infusion rates, pharmocologic agents, dosage, and time for initiation of treatment have yet to be determined.

A D dimer biosensor could provide feedback on the dosage and infusion rate needed to lyse the clot. D dimer sensors could help eliminate the guesswork related to the dosage and infusion rate of the thrombolytic agents, providing surgeons with a faster diagnosis and treatment plan and reducing hemorrhaging incidents.

Sensor Fabrication and Optical System

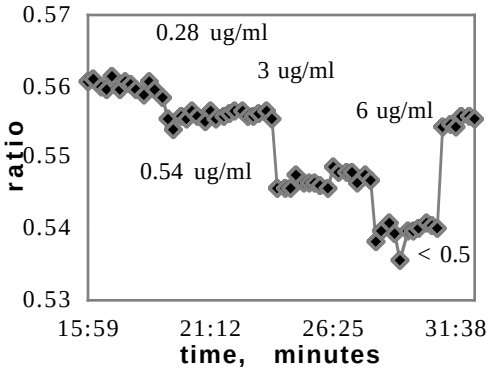
D dimer monoclonal antibodies with a fluorescein isothiocyanate (FITC) label were physically immobilized on the end of the 125 μm silica optical fiber using sol-gel dip coating methods. To prepare the sol-gel solutions used in dipcoating the optical fibers, 0.5 ml TMOS, 1.0 ml deionized water, and 10 μl 0.04 M HCl were sonicated in an ice bath for approximately 30 minutes. To 0.5 ml of this solution was added 1.0 ml PBS. D dimer-FITC antibodies were added to this solution and fiber tips were immediately dipcoated. The D dimer concentration was 1.125 μM.

For the D dimer sensor, a ratiometric-based system was utilized. Excitation was provided by a tungsten halogen lamp. This light passed through a chopper wheel (1 kHz) and then was split with some directed into a reference photosensitive module. The rest of the light passed through a biconvex lens and an interference filter. It was then reflected by a dichroic mirror. It passed through a second biconvex lens which coupled the light to the optical fiber. The emission fluorescence traveled back through the fiber and biconvex lens and was transmitted through the dichroic mirror, filtered and then coupled to a second photosensitive module. The photosensitve modules and chopper control were interfaced to lock-in amplifiers which were interfaced to a computer. A computer program then calculated the voltage ratio of the two photosensitve modules.

Results and Discussion

The D dimer antibodies encapsulated within a silica sol-gel matrix on the tip of an optical fiber definitely shows an affinity for its corresponding antigen as shown in Figure 1. In Figure 1, the response of the D dimer sensor is shown when immerse in 2 ml of healthy human blood. Since the presence of D dimer antigens will quench the FITC fluorescence, (+) control D dimer antigens were added to the 2 ml of blood in 100 μl increments in order to determine a minimum detection level for this particular sensor. A decrease in fluorescence was first recorded when 200 μl of D dimer antigens were added to 2 ml of blood which gave a D dimer concentration of 0.54 μg/ml in the solution. Additional decreases in fluorescence were recorded up to a D dimer antigen concentration of 6 μg/ml. Reversibility was tested by diluting the solution to obtain a D dimer antigen concentration of less than 0.5 μg/ml in solution. There was a regain in fluorescence but not to the original intensity.

Figure 1. D dimer sensor



The feasibility of a D dimer sensor has been established; however, some improvements on the D dimer antibody encapsulation method are necessary. Initial studies indicated that the D dimer sensor is not completely reversible. The high affinity between the D dimer antibodies and its antigens is responsible for the sluggish and incomplete reversibility. Studies are underway to improve the reversibility by modifying the encapsulation method which includes the addition of surfactants to the sol-gel precursor materials.

References

1. JC Grotta, et al.;*Stroke:Clinical Updates*. 3:9 (1994)

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